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SERUM AND SPUTUM SURFACTANTS -A AND -D IN MULTIDRUG-RESISTANT AND DRUG-SENSITIVE TUBERCULOSIS PATIENTS

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ABSTRACT

Abnormal production and function of surfactants are associated with pulmonary diseases. Also, pulmonary infections alter surfactant metabolism. Due to lack of information on the levels of surfactants A (SP-A) and D (SP-D) in Nigerian tuberculosis (TB) patients, this study assessed these surfactants in both sputum and serum of drug sensitive- and multidrug resistant- TB patients using ELISA. The aim is to explore the diagnostic or therapeutic potential of SP-A and SP-D in TB patients. Also, to find out appropriate sample for the analyses of SP-A and SP-D in TB patients.

The mean serum levels of SP-A and SP-D were not significantly reduced in MDR-TB (n=30) and DS-TB patients (n=30) compared with non-TB apparently healthy controls (n=30) ($p > 0.05$). Mean sputum levels of SP-A and SP-D were significantly reduced in DS-TB patients compared with the levels in MDR-TB patients ($p < 0.05$). These findings suggest that the sputum SP-D and SP-A levels but not serum SP-D and SP-A levels are useful indicators of the disease activity in pulmonary TB patients.

Keywords: Tuberculosis, Surfactants, Diagnosis, Nigeria

LES TAUX SÉRIQUES D'EXPECTORATIONS ET SANS TENSIOACTIFS- A ET D DANS LA TUBERCULOSE MULTIRÉSISTANTE ET LA TUBERCULOSE CHEZ LES PATIENTS SENSIBLES

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Résumé

production anormale et la fonction d'agents tensio-actifs sont associés à des maladies pulmonaires. En outre, les infections pulmonaires modifier le métabolisme du surfactant. En raison du manque d'informations sur les niveaux d'agents de surface A (SP-A) et D (SP-D) in Nigerian la tuberculose (TB) patients, cette étude a évalué ces surfactants dans les expectorations et le sérum de la multirésistance et sensible- patients atteints de tuberculose à l'aide d'ELISA. L'objectif est d'explorer les possibilités diagnostiques ou thérapeutiques de SP-A et SP-D dans les patients atteints de tuberculose. Aussi, nous avons voulu en savoir échantillon approprié pour l'analyse de SP-A et SP-D dans les patients atteints de tuberculose.

Les niveaux sériques de SP-A et SP-D n'ont pas été significativement réduite dans la TB-MR (n =30) et DS-TB patients (n =30) par rapport aux non-contrôles apparemment en bonne santé (n =30) ($p > 0,05$). Les niveaux d'expectorations de SP-A et SP-D ont été significativement réduits dans DS-tuberculeux par rapport aux niveaux de la multirésistance patients ($p < 0,05$). Ces résultats suggèrent que l'expectoration SP-D et SP-A mais pas la SP-D et SP-A sont des indicateurs utiles à l'activité de la maladie chez les patients atteints de tuberculose pulmonaire.

Mots clés : Tuberculose, surfactants, le diagnostic, le Nigeria

INTRODUCTION

Mycobacterium tuberculosis (*M.tb*) is a facultative intracellular pathogen that infects humans causing tuberculosis (1, 2). Inhalation of infectious aerosols is the common route of transmission and it is finally deposited in the terminal bronchioles and alveoli of the lungs (3). At this early stage of infection, interactions of *M.tb* with the innate immune environment of the lung have been proposed to determine the outcome and progression of *M.tb* infection (2). However, such interactions are

incompletely known. One of the first interactions of *M.tb* in the lung is the binding is pulmonary surfactant molecules, which are multi-molecular complexes composed of lipids and proteins secreted by alveolar type II cells and Clara cells in distal bronchioles (4, 5).

Four specific surfactant proteins have been identified viz: surfactant protein SP-A, SP-B, SP-C and SP-D. SP-B and SP-C have been characterized as hydrophobic polypeptides that enhance the adsorption of lipid to the surface of the alveoli (6).

On the other hand SP-A and SP-D are hydrophilic and participate in the innate host defense immune system. SP-D binds to macrophages and neutrophils and promotes phagocytosis and killing of bacteria, fungi, and viruses (7). SP-A and SP-D lines the lungs and are essential for pulmonary function by reducing surface tension (5-7). SP-A and SP-D also play important roles in innate immune responses to a wide range of respiratory pathogens including *M.tb* (8-15). Additionally, it has been shown that pulmonary SP-D contributes significantly to host control of infections by the parasitic helminth *Nippostrongylus brasiliensis* (16).

Binding of pathogens by SP-A and SP-D trigger various immune responses, including opsonisation leading to enhanced phagocytosis and killing by recruited macrophages and neutrophils via oxidative mechanisms, aggregation of pathogens thereby hindering their entry into host cells, and direct microbicidal activities by increasing cellular membrane permeability (17). SPs also assist in the clearance of apoptotic cells and in modulating inflammation (18). The interaction of SPs with immune cells to initiate clearance mechanisms is mediated by SP's collagen region with a number of proposed receptor molecules on these cells (19).

Considering the intricate relationship between SP-A and SP-D with innate immune cells necessary to control respiratory pathogens, the present study thus determined both serum and sputum levels of SP-A and SP-D in DS-TB and MDR-TB, which were not previously studied among Nigerian TB patients.

MATERIALS AND METHODS

A total of 90 participants were enrolled for this study. This comprised of thirty (30) multi-drug resistant TB (MDR-TB) patients, thirty (30) drug-sensitive TB (DS-TB) patients and thirty (30) non-TB apparently healthy controls. MDR-TB patients had been previously diagnosed as being infected with isoniazid and rifampicin resistant strains of *Mycobacterium tuberculosis* (Mtb) using clinical history, Chest X-ray and GENE Xpert. These patients were admitted into the MDR-TB centre,

University College Hospital (UCH) Ibadan, Nigeria for anti-TB treatment. DS-TB patients were recruited from the Medicine Out-patient Clinic, University College Hospital, Ibadan, Nigeria by a Consultant Chest Physician after confirmation with Microbiological test (sputum smear microscopy), chest X-ray and clinical history. Five milliliters (5 ml) of blood was drawn from the antecubital vein of each participant and dispensed into sterile plain sample tubes without anticoagulant. Blood was centrifuged at 1000g for 15mins and serum was obtained. Serum was stored at -20°C until analysis. Spot collection of sputum sample was carried out. Study participants were asked to rinse their mouth with water to reduce salivary contamination of sputum. Sputum produced was collected into 20ml sterile disposable polypropylene tubes and kept on ice from collection to arrival at the laboratory. Sputum sample was homogenized with an equal volume of phosphate buffered saline (PBS). Homogenate was centrifuged at 10,000g for 10 minutes to remove cellular debris and mucus, and supernatant obtained was stored at -20°C until analysis. Enzyme Linked Immunosorbent Assay (ELISA) method was used for the determination of serum and sputum concentrations of surfactant-associated protein A (SP-A) and Surfactant-associated protein D (SP-D) as previously carried out (20).

RESULTS

As shown in the Table, the mean serum levels of SP-A were not significantly reduced in MDR-TB patients and DS-TB patients compared with control while the mean serum levels of SP-D were not significantly raised in MDR-TB patients and DS-TB patients compared with control. The mean sputum levels of SP-A was significantly reduced in DS-TB patients compared with MDR-TB patients and control while the mean sputum levels of SP-D were significantly raised in DS-TB patients and MDR-TB patients compared with control. Also, the mean sputum level of SP-D was significantly raised in MDR-TB patients compared with DS-TB patients.

TABLE 1: COMPARISON OF MEAN LEVELS OF SPUTUM AND SERUM SP-A (NG/L) AND SP-D (PG/ML) IN MDR-TB, DS-TB AND CONTROL

Variables	MDR-TB	DS-TB	Control	p'	p''	p'''
Serum SP-A	306.90±133.32	344.66±139.87	368.72±210.37	0.361	0.725	0.455
Sputum SP-A	503.22±170.25	312.03±187.98	570.15±175.43	0.326	0.001*	0.007*
Serum SP-D	1709.38±665.56	1701.31±596.96	1547.34±324.58	0.448	0.430	0.972
Sputum SP-D	3596.45±636.45	2600.0±1085.04	1210.57±457.39	0.000*	0.000*	0.005*

p' MDR-TB compared with control; p'' DS-TB compared with control; p''' MDR-TB compared with DS-TB

*Significant at p<0.05

DISCUSSION

Tuberculosis which claims at least 3 million lives annually is caused by *M.tb* (1, 2). The interaction between *M.tb* and the pulmonary lining represents the initial contact of the bacterium with the host immune system (1-3), thus the factors which affect phagocytosis of *M.tb* by host immune cells are important to our understanding of disease pathogenesis. Surfactant and its associated proteins, in conjunction with alveolar macrophages, are important components of the pulmonary alveoli. Surfactant proteins (SP-A, SP-B, SP-C, and SP-D) are enriched with a relatively unique phospholipid (dipalmitoylphosphatidylcholine) (3, 4). The main functions of surfactant are lowering surface tension at the air-liquid interface and thus preventing alveolar collapse at end-expiration, interacting with and subsequent killing of pathogens or preventing their dissemination and modulating immune responses (5-7). Two major surfactant associated proteins, surfactant protein A (SP-A) and surfactant protein D (SP-D), contain carbohydrate recognition domains (CRDs) (3-7).

SP-A binds with lipopolysaccharides and peptidoglycan derived from *K. pneumoniae*, *E. coli*, *P. aeruginosa* and *Legionella pneumophila*, *Staphylococcus aureus* and *Streptococcus pneumoniae* as well as *Mycobacterium avium*, *Mycobacterium tuberculosis* and *Mycoplasma pneumoniae* which consequently result in agglutination, enhancement of pathogen uptake, and growth inhibition (8-15). The metabolism of SP-A in TB patients considered for this study might account for reduced levels of SP-A especially in the sputum which is a transmission medium for *M.tb*. This may be to agglutinate *M.tb* in local environment. This hypothesis is supported by a previous study (21) which reported that human SP-A enhances the uptake of the virulent Erdman strain of *M.tb* by human monocyte-derived macrophages and human alveolar macrophages through direct interaction with the macrophages. The study thus concluded that SP-A was reported to play a role in the pathogenesis of tuberculosis by enhancing the ability of *M.tb* to enter and survive within its local niche in the host. Another data suggested that SP-A has pleiotropic effects even at the low concentrations found in tuberculosis patients (22). SP-A was reported to augment inflammation in the presence of infection and inhibits inflammation in uninfected macrophages so as to protect uninvolved lung segments from the deleterious effects of inflammation (23). This further support reduced level of SP-A in TB patients as a result of continuous metabolism.

SP-D is synthesized and secreted into the airspaces of the lung by the respiratory epithelium (4, 5). At the alveolar level, SP-D is constitutively synthesized and secreted by alveolar type II cells. More

proximally in the lung, SP-D is secreted by a subset of bronchiolar epithelial cells, the non-ciliated Clara cells. Because SP-D is stored within the secretory granules of Clara cells (6, 9), it seems likely that SP-D is subject to regulated secretion via granule exocytosis at this level of the respiratory tract. In some species, SP-D is also synthesized by epithelial cells and/or submucosal glands associated with the bronchi and trachea (6). This might explain significantly raised sputum levels of SP-D especially in TB patients. SP-D has been reported to agglutinate bacteria, delay phagocytosis and facilitate phagolysosomal fusion (10-12). This explains why SP-D was higher in MDR-TB patients than DS-TB patients considered for this study.

The opposing trends of sputum SP-A (reduced level) and sputum SP-D (raised level) observed in TB patients considered for our study might be related to slight differences in their chemical composition and functions. SP-A preferentially binds monosaccharides and lipid ligands, while SP-D additionally binds disaccharides, complex carbohydrates and anionic phospholipids present on cell surfaces (18). This might be explained by subtle structural differences between the CRDs of trimeric SP-A and SP-D resulting in the flatter and more hydrophobic surface of SP-A preferentially binding to less polar substrates, while the more hydrophilic surface of SP-D displays a higher affinity for highly polar targets (24). Moreover, in SP-A, the Gly-X-Y repeats in the collagenous region are interrupted resulting in a kink (and hence the bunch-like structure) and a relatively smaller distance to the distal CRD compared to SP-D which displays more freedom to bind and aggregate target pathogens (25).

In the case of *M. tuberculosis*, SP-D has been shown to bind mannosylated lipoarabinomannan (ManLam) from the Erdman strain (26), while SP-A has been shown to bind a wider range of mycobacterial targets, including ManLam (from virulent and avirulent mycobacterial strains) (27), lipomannan (28), a 60-kDa glycoprotein (29), and the *M. tuberculosis* surface glycoprotein Apa (30). In addition to their carbohydrate specificities, SP-A and SP-D was also reported to differ with respect to several other structural features that determine their interactions with the innate immune system. SP-A forms a bouquet-like 18-mer that associates with surfactant lipids and tubular myosin and can bind to the C1qR receptor, toll-like receptors TLR2 and TLR4, the CD91/calreticulin complex, the signal inhibitory regulatory protein SIRP- α , and the unconventional myosin XVIIIa receptor SPR210. SP-D, on the other hand, forms a cross-like dodecamer (12 chains) that resides in the aqueous phase of the alveoli and can bind microfibril-associated protein 4, CD14, defensins, decorin, C1q, TLR2, TLR4, and a 340-kDa glycoprotein of unknown function (31).

Exploration of surfactant A therapeutic potential is proposed by the present authors. Animal study and limited human data show that exogenous surfactant decreases DNA synthesis of inflammatory mediators (32, 33). Intratracheal administration of a surfactant-amikacin mixture to rats with *Pseudomonas pneumonia* showed improved anti-inflammatory effects compared with amikacin alone (33). There is also evidence that certain pharmacologic agents may enhance endogenous surfactant levels, although the current data are limited. Antenatal steroids accelerate development

of type 2 pneumocytes and thus increase the production of surfactant proteins and enzymes necessary for phospholipid synthesis. Corticosteroids also induce pulmonary β -receptors, which play a role in surfactant release and alveolar fluid absorption when stimulated (34). These observations suggest the possibility that surfactant or its stimulants be used to modulate immune responses during inflammatory lung disease like tuberculosis, but further studies are necessary.

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